# A PROXIMATE ANALYSIS OF THE ALCOHOLIC EXTRACT OF THE ROOT OF RUMEX CRISPUS, AND A COMPARISON OF THE HYDROXY—METHYL—ANTHRAQUINONES PRESENT WITH THOSE FROM CERTAIN OTHER DRUGS

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY

SUPERVISION BY Ruth E. Okey

ENTITLED A Proximate Analysis of the Alcoholic Extract of the Root of Rumex crispus, and a Comparison of the Hydroxy-methyl-anthraquinones Present with Those from Certain Other Drugs.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR

THE DEGREE OF Doctor of Philosophy in Chemistry.

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## I. Statement of Problem:

Rumex crispus, usually known as yellow dock, is one of the commonest and most troublesome of American weeds, being widely distributed over the country. In 1890, the dried root and the fluidextract were official in the United States Pharmacopoeia, and at the present time the drug seems to have a rather wide use in proprietary medicines.

Since there was abundant evidence in the literature that some varieties of Rumex contained hydroxy-methyl-anthraquinones, notably emodin and chrysophanic acid, and the composition of this variety was practically undetermined, the present study was undertaken. If yellow dock contains emodin and chrysophanic acid in appreciable quantities, it will undoubtedly furnish a cheaper source of these substances than cascara and aloes, with which it is compared in this paper.

## II Historical.

### A. Survey of the Composition of Various Varieties of Rumex.

The plants containing hydroxy-methyl derivatives of anthraquinone have, since the eighteenth century, attracted the attention of many chemists. Long before anything definite was known about the chemical composition of these substances, it was generally conceded that rhubarb, frangula , aloes, senna and some varieties of rumex contained yellow dyestuffs which were either chemically identical, or very similar in nature. Previously to 1840, various anthraquinone containing materials known as "rhein", "rhabarbergelb", "chrysophanic acid", etc., had been described. Some of the more interesting of these early papers are listed below:

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Geiger, "Über den Rhabarbergelb", Ann. Chem. Pharm. 9, 91,1834.
O. Henry, "Untersuchung der Wurzel von Rheum australe", Pharm. Centralbl., 1836, 631.
Buchner, "Resulte der bisherige Untersuchungen über Rhabarber"., Repert. Pharm. 65, 126, 1837.
Brandes and Leber, "Zusammensetzen der Rhabarbersäure", Arch.d. Pharm. xvii, 42, 1839.

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Each of these earlier workers described a more or less different product, and each seemed to be perfectly sure that his own preparations were pure substances, while those of other investigators, who had used different methods of preparation, were mixtures. In 1845, Schlossberger and Doepping ("Chemische Untersuchung der Rhabarberwurzel", Ann. 50, 196) exclaim, "Gegen zwanzig einzelne Arbeiten, zum Theil sehr tüchtiger Chemiker, über einem und denselben Gegenstand, und doch einigermaszen aufklärende Untersuchungen kaum angedeutet!" And, in fact, practically all of the analyses made within the next fifty

years were to fail to give satisfactory evidence of the composition of the yellow dyestuff of rhubarb. The nearer they approach to real knowledge of the complexity of plant products, the slower chemists are in making exact statements as to the nature of such substances.

The analyses of the various varieties of Rumex recorded in the literature since 1830 may be reviewed briefly as follows:

In 1831, Buchner and Herberger (Repert. Pharm. 38, 337.) prepared a "rumicin" by extracting the roots of Rumex obtusifolius with ether, and following this by an extraction with alcohol. The so-called "rumicin" was separated from the alcoholic extract, and was so impure, according to Thann, that the red color with alkalies was not even noticed.

L.F. Bley, in 1833, (Trommd. N. Jour. 25, ser. 2, 68.) studied the composition of Rumex acutus, obtaining fats, resins, oils, etc., but no definite chemical substances.

An investigation made by Geiger, (Ann. Chem. Pharm. 9, 304, 1834 resulted in the isolation of another anthra-quinone containing product, also known as "rumicin". Geiger extracted the root with alcohol, precipitated the concentrated extract with water, and extracted this precipitate with ether. The ether extract, when finally washed with alcohol, yielded the yellowish, non-crystalline powder which he called "rumicin".

Riegel (Jahr. pr. Pharm. 4, 72 & 129, 1841) prepared another "rumicin" by extracting the roots of Rumex obtusifolius with ether and recrystallizing from alcohol the residue left after removing the ether.

Karl von Thann (Wien. Acad. Ber. 31, 26, and Am. Jour. Pharm. 31, 152, 1858.) made a study of this "rumicin" from Rumex

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obtusifolius; making use, for the first time, of Rochleder and Heldt's purification by means of solution in ammonia, followed by precipitation with acetic acid. He obtained his mixture of anthraquinone derivatives in crystalline form, and described their reactions toward the ordinary laboratory solvents and precipitants in great detail. He noted especially the reddish purple color with alkalies, becoming darker on exposure to air, and the property of sublimation, which is possessed in an extraordinary degree by these substances. He considered this "rumicin" identical with the substance then known as "chrysophanic acid" from rhubarb.

Following this work, H. Grothe (Pogg. Ann. cxiii, 190, and Jahr. 14, 707, 1861.) isolated a substance which he called "chrysophanic acid" from Rumex pyramidalis, R. palustris, R acutus, R. aquaticus, and R. hydrolapathium. His methods were those of Schlossberger and Doepping. (loc.cit.)

The next investigation, that of A. Hilger, (Landw. Vers. Stat. 23, 456, 1879.) dealt with the absorbtion spectra of the yellow coloring matter of Rumex acetosa. In 1885, Bertholet and Andre (Compt. rend. 101, 354, 1885) reported the existence of oxalic acid in Rumex acetosa.

O. Hesse (Ann. 291, 305, 1896) made an analysis of Rumex nepalensis. He considered the dihydroxy methyl anthraquinone derivative present, which he called "rumicin" to be isomeric with and very similar to chrysophanic acid. He described it as melting at 186-188°C, soluble in potassium hydroxide, and precipitated from this solution by means of carbon dioxide. This substance seems, in the light of our present knowledge,

to have been a mixture of emodin-mono-methyl-ether and chrysophanic acid. The other active constituents he describes are nepalin, $C_{17}H_{14}O_4$, crystallizing in microscopic needles, m.p. 136° from glacial acetic acid, insoluble in alkali carbonates and soluble in alkali hydroxides with a purple color, yielding a diacetyl derivative melting at 171°, after darkening at 170°; and nepodin, which crystallizes from benzene and ligroin in greenish yellow prisms, m.p. 156°, is soluble in alkali carbonates, gives a red solution in concentrated sulfuric acid, and a diacetyl derivative which darkens at 180° and melts at 198°.

The latest, and by far the most valuable work on a plant of the Rumex family, is that of Tutin and Clewer (J. Ch. Soc. 97, 1, 1910.) on Rumex ecklonianus. This variety is native to South Africa, and the material used for the analysis was the dried overground portion of the herb. The investigation followed the usual lines of plant analyses made in the Wellcome Research laboratories, and was, in the main outline, very much on the order of the present investigation.

This study resulted in the identification of the following substances: ceryl alcohol, a phytosterol, various fatty acids, ipuranol, kaempferol, emodin, chrysophanic acid, emodin-mono-methyl-ether, and a sugar which yielded d-phenyl glucosazone.

So far as the literature indicates, no modern scientific investigation of the composition of Rumex crispus has been carried out up to the present time.

B. Composition of the active Constituents.

Before the discovery of emodin by De la Rue and Miller (Am. Jour. Pharm. 30, 442-447, 1858.) practically no pure substances had been isolated from the hydroxy-methyl-anthraquinone containing material present in any plants. The name, "chrysophanic acid", or "chrysophane",-- meaning "I appear gold"-- had been applied loosely to any coloring matter of this type; and it was not until 1905 that the substance now known as chrysophanic acid was isolated in pure state. (Oesterle, Arch. d. Pharm. 243, 434.)

Schmidt, in 1875, (Ber. 8, 1275.) offered the first real evidence of the structure of these compounds. He distilled aloes with zinc dust, obtaining a substance which he was able to prove was a methyl anthracene. During the next few years, Liebermann published several papers dealing with the structure of the various naturally occurring hydroxy-methyl-anthraquinone dyestuffs. In 1877 (Ann. 138, 145-224.) he identified emodin as a tri-hydroxy methyl anthraquinone, and chrysophanic acid as a dihydroxy methyl anthraquinone.

Liebermann considered the hydrocarbon of Schmidt to be $\beta$-methyl anthracene, but Jowett and Potter (J. Ch. Soc. 83, 1327, 1903.) questioned this structure; without, however, offering any very good foundation for their views. This was also true of the work of Perkin. (J. Ch. Soc. 65, 925, 1894.)

In 1911, Fischer and Sapper (J. pr. Chem. 83, 203.) prepared a known $\alpha$-methyl anthracene by distilling 1-4 chloro-methyl anthraquinone with zinc dust. This compound differed so completely in its properties from the methyl anthracene of aloe-emodin that this latter compound had to be considered a $\beta$-methyl anthracene.

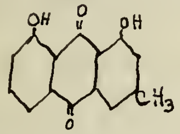
In the same year, Fischer, Falco, and Gross (J. pr. Chem. 83, 208) proved that the anthracene from chrysophanic acid was a β-methyl anthracene by converting its zinc dust reduction product to a β-methyl anthraquinone, the structure of which had been proven by synthesis.

In 1910 Oesterle (Arch. d. Pharm. 248, 476.) showed that the substance accompanying chrysophanic acid in most plants, and having the same solubilities as chrysophanic acid, was the monomethyl ether of frangula emodin. He found, at this time, that his former method of removing the methoxyl group from this substance by the action of anhydrous aluminium chloride was not so satisfactory as merely heating the mixture in solution in concentrated sulfuric acid to 160°.

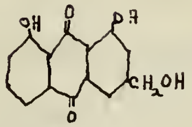
In 1911, the same author, (Arch. d. Pharm. 249, 445) showed that on reduction, aloe-emodin gave chrysophan hydranthon, and that on oxidation with air in the presence of sodium hydroxide, this gave chrysophanic acid. Since he had already shown (Schw. Woch. Chem. Pharm., 1903, No. 50.) that rhëin might be made by oxidizing aloe-emodin or chrysophanic acid, this completed the evidence that chrysophanic acid, aloe-emodin and rhēin are different stages in the oxidation of the same hydroxy methyl anthraquinone, and that the $CH_3$, the $CH_2OH$, and the COOH groups are in the β position.

Later investigation by the same man (Arch. d. Pharm. 250, 301) indicates that this position is the "3" rather than the "2" position, assuming that one OH group is in the "1" position. Oesterle has also done a great deal of work with a view to the determination of the exact placing of the OH groups. Not all of

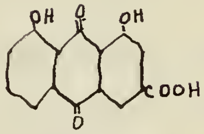
this is conclusive, but the best evidence points to the following formulae:



Chrysophanic Acid

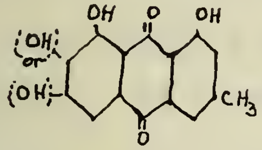


Aloe-emodin



Rhein.

He considers, also, that since frangula emodin is so often associated with chrysophanic acid in plant tissues, it is probable that both contain the same dihydroxy-methyl-anthraquinone nucleus, while the behavior of the tetra nitro compound of emodin indicates that the third hydroxyl group must be in the "6" or the "7" position, giving the following formula:



Emodin.

The physical and chemical properties of these substances will be discussed in the part of the thesis dealing with the experimental work.

## III. Experimental.

### A. General Methods of Analysis.

The type of analysis attempted was, in general, much like that used in the Wellcome Research Laboratories for medicinal plants. In several places it was necessary to use more than one method of separation; and in some cases a number of different methods were tried out with only a moderate amount of success. An outline of the separation follows:

A preliminary examination was made by exhausting a weighed portion of the drug successively with petroleum ether, ethyl ether, chloroform, ethyl acetate and alcohol; distilling off the solvents and weighing the material extracted.

The drug was then exhausted by percolation with cold 95% alcohol. This extract, after concentration under diminished pressure to the consistency of a thick syrup, was poured into a large excess of water, and the precipitated resins filtered off and washed with cold water.

The water soluble fraction was usually shaken with several times its volume of ether, and sometimes this was followed by a similar extraction with amyl alcohol. The aqueous layer remaining was then precipitated with neutral lead acetate, and the precipitate decomposed by hydrogen sulfide, the excess lead being removed from the filtrate by the same means. The lead acetate precipitate should contain, besides large amounts of resins, the lead salts of plant acids soluble in alcohol and water, while the filtrate from this precipitate should contain the soluble carbohydrates. The acids and sugars have to be separated by special means.

The ether and amyl alcohol extracts of the water solutions should contain the substances relatively more soluble in organic solvents than in water. So these extracts were shaken successively with approximately 8% ammonium carbonate solution, 2 to 5% sodium carbonate, and 2% sodium hydroxide;removing respectively the strong acids, the weaker acids, and the phenolic substances.

Emodin, as a trihydroxy methyl anthraquinone, is soluble in sodium carbonate solutions, while the dihydroxy methyl anthraquinone, chrysophanic acid, and emodin-mono-methyl-ether require an alkaline hydroxide for their extraction. Substances of an alkaline nature, such as alkaloids and amines, were removed by shaking the immiscible solvent extracts with acids of varying strengths.

The neutral substances, if any are present after washing with alkalies and acids, should include the alcohols of higher molecular weight which are still water soluble. In dealing with the drugs investigated, it was usually found that acid soluble substances were absent, while neutral substances were present only in traces.

The water insoluble resins were dried at as low a temperature as possible, mixed with purified sawdust, and extracted in a Soxhlet apparatus successively with petroleum ether, ether, chloroform, and sometimes with ethyl acetate and alcohol.

The petroleum ether was usually distilled off, and the residues from this solution taken up with ethyl ether. If any very great amount of fats or fatty acids was present, as was usually the case in the analysis of a plant of this sort, the

direct shaking out with alkalies was very much complicated by the formation of emulsions. So, in one case, this method was modified by saponifying the entire petroleum ether soluble fraction with alcoholic potash, distilling off the alcohol, and extracting the neutral substances, in this case a phytosterol and a hydrocarbon, with dry ether. The whole was then acidified and shaken out with ether. this ether solution was then shaken successively with ammonium carbonate, sodium carbonate and sodium hydroxide solutions. The fatty acids extracted by the sodium carbonate were esterified, and the esters fractionally distilled. The emodin had been removed previously by shaking the ether solutions of the esters with alkalies. In this case, the sodium hydroxide extract of the acidic material that was set free by adding acid to the alkaline solutions from the original saponification yielded almost pure chrysophanic acid.

The ethyl ether extract of the water insoluble resins was shaken out directly with the different alkalies. The sodium carbonate soluble fraction of this extract contained the bulk of the emodin, while the sodium hydroxide extract yielded fairly large amounts of a mixture of chrysophanic acid and emodin-mono-methyl-ether.

The chloroform and alcohol extracts of the water insoluble material were always resinous in nature.

B. Apparatus.

While no especially original or extraordinary apparatus was used in this investigation, it seems worth while to mention some ways in which ordinary laboratory apparatus has been adapted to our purposes.

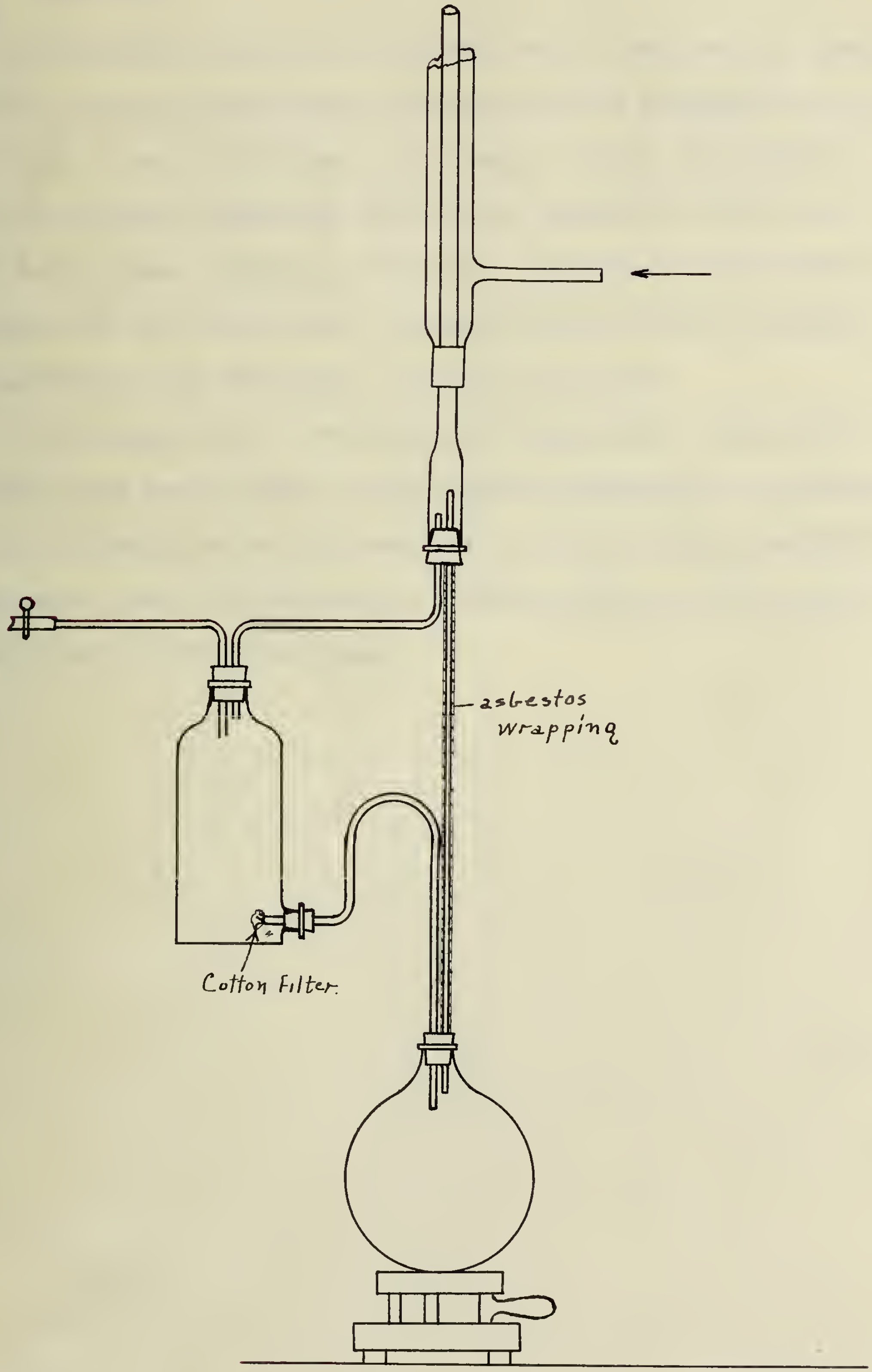
The percolators used in the original extraction of the drug were made from twelve and sixteen liter bottles, the bottoms of which had been cut off. These were inverted, and fitted with rubber stoppers and tubes in the usual way.

Some of the extractions of the water insoluble resins were carried out in a continuous extraction apparatus designed by Dr. D.F. McFarland of the Division of Industrial Chemistry. A sketch of this apparatus is inserted with his permission.

Where the quantities of material to be extracted by shaking with immiscible solvents were so large as to render handling in an ordinary separatory funnel impracticable, these extractions were made in twelve liter bottles. These were of such a shape as to be capable of insertion in the place of the stone jars on a motor driven ball mill. This provided for a slow rotation of the two layers of liquid until equilibrium was established. Where emulsification was a large factor, this type of extraction was more successful than shaking in an ordinary separatory funnel.

For small quantities of solution with marked tendencies toward emulsification, the hour glass type of separatory funnel suggested by C.H. LaWall, (Jour. Am. Pharm. Assoc. 3, 498-99, 1914.) consisting of two bulbs with a somewhat constricted opening between them, proved very useful.

For the purpose of drying fatty acids and resinous residues which seemed to change composition on exposure to air, a large vacuum desiccator was used. This was so arranged that a current of carbon dioxide could be passed in at the bottom and out at the top, and was heated by a twenty five watt carbon filament electric bulb.



McFarland Extraction Apparatus Plate I

## C. Analysis of Rumex crispus.

### I. Material.

The material used for analysis was: Twenty-five pounds of the dried and powdered root of Rumex crispus obtained in the fall of 1915 from Meyer Bros., St. Louis; about ten pounds of the root of this plant collected during the summers of 1916 and 1917 in Warren and Champaign counties, Illinois; and a fifty pound sample of the dried root obtained in the form of small pieces from Fuller and Morrison, Chicago, in 1917.

The analyses are not reported separately, since the first sample was used almost entirely for preliminary experiments with methods for the preparation of pure hydroxy-methyl-anthraquinones, and the substances isolated during this work were also isolated in later analyses.

## II. Preliminary Examination of the Drug.

Fifty grams of the dried root in No. 80 powder were exhausted in a Soxhlet apparatus with the following succession of solvents:

| | | | | |
|---|---|---|---|---|
| 1. Petroleum ether | dissolved | 0.5356% | by | weight. |
| 2. Ethyl ether | " | 0.4242% | " | " |
| 3. Chloroform | " | 0.5382% | " | " |
| 4. Ethyl acetate | " | 0.7168% | " | " |
| 5. Alcohol | " | over 3% | " | " . |

All of these extracts gave very deep red colors with alkalies; indicating the presence of hydroxy-methyl-anthraquinones, either in the free state or as glucosidic resins.

Sections of the freshly dug roots of Rumex crispus were treated with ten per cent sodium hydroxide. The first part of the root to show the red stain was a circle directly inside the bark, but within thirty minutes the entire cut surface was almost uniformly red.

## III. Systematic Analysis.

The drug was ground as finely as possible (20 to 30 powder), packed in percolators of the type described above, and exhausted by percolation with cold 95% alcohol. The alcoholic extracts so obtained were concentrated under diminished pressure until they formed a thick viscid liquid, in the proportion of about one gram for twenty to thirty grams of the drug. This alcoholic concentrate was then precipitated by pouring it into a large excess of water; separating it into a water soluble extract, and a water insoluble resinous precipitate.

### (1) The Water Soluble Extract.

(a) A preliminary examination of a portion of this water soluble extract was carried out by shaking it with a large amount of ether, and following this by precipitation of the material

remaining in aqueous solution successively with neutral and basic lead acetates. This resulted, after the removal of the lead as lead sulfide, in the separation of much resinous material. Nothing crystalline could be isolated from either precipitate. A positive reaction was obtained with Molisch's reagent for sugars after the removal of the excess lead from the basic lead acetate precipitate, but no osazone was isolated.

The ether extract of the original water soluble fraction was light yellow in color. On shaking it with the various alkalies, and acidifying the extracts so obtained, only very small quantities of impure emodin were isolated.

(b) A second portion of this aqueous extract was treated with hydrochloric acid, at first only to distinct acid reaction, when considerable turbidity resulted. The addition of more acid-- up to a concentration of 5% actual hydrochloric acid--was found to increase the amount of precipitate formed.

The precipitate so obtained was filtered off, and the filtrate allowed to stand overnight in large glass bottles. A second precipitate was found to have settled out by morning. This was filtered off and added to the first. The only plausible explanation of this second precipitation appears to be the hydrolysis of some glucosidic material by the prolonged action of the acid.

These precipitates were combined and examined together with similar precipitates obtained later on by adding sulfuric acid to another portion of the original water soluble fraction. The weight of this precipitate, after drying, was about one gram from ten pounds of the drug. Its bulk was so great, however,

that it seemed to be much larger in amount.

The acid filtrate was extracted by shaking successively with petroleum ether, ethyl ether, and amyl alcohol (represented by the fusel oil fraction boiling between 128° and 132° C.)

The petroleum ether extract was very light in color, and yielded practically no residue on distillation.

The ethyl ether extract was also very light yellow in color and gave a residue too small in amount for investigation.

The extraction with amyl alcohol was repeated three times, and the dark reddish brown extracts were combined. This amyl alcohol solution was washed many times with a large excess of ammonium carbonate, and these extracts acidified with hydrochloric acid. They remained a deep red in color. Finally, the emulsions which had formed in the layer between the amyl alcohol and the carbonate solution were allowed to go in with the last portions of the carbonate extracts. The top of this mixture, containing the emulsions, a certain amount of a reddish brown precipitate which had formed on acidification of the carbonate, and some amyl alcohol held mechanically, was transferred to a four liter flask, in order to remove the amyl alcohol by steam distillation under diminished pressure.

As the distillation proceeded, the mixture in the flask became deeper and deeper red in color, until finally the amyl alcohol layer in the distilling flask had about the general appearance of red ink. After all the amyl alcohol had distilled over, the liquid in the flask was filtered while hot. A large quantity (about twenty grams) of a very porous reddish precipitate was obtained, of which roughly 10-12% was soluble in ethyl

alcohol with a very deep red color.

The residue obtained on evaporation of the alcohol from this solution of the pigment formed non crystalline scales. It was very slightly soluble in water, and on the addition of small amounts of alkalies, turned green. As the concentration of the alkali was increased, however, it became blackish, and then with still more alkali, almost as deep a red as it had been in the original acid solution.

On elementary analysis by fusion with sodium in the usual way, traces of nitrogen were found to be present, but no sulfur, and only traces of halogen which were to be accounted for as occluded chloride.

The alcohol soluble pigment was slightly soluble in ether, and entirely insoluble in chloroform. Potassium permanganate in acid solution was decolorized by the pigment. With hydrogen peroxide, no effect was noted. Formaldehyde lightened the red color perceptibly. Nitric acid produced a lighter and more brownish red shade, while concentrated sulfuric acid gave a deep brown color. In alcoholic solution the pigment was not precipitated by silver nitrate or barium chloride, while ferric chloride gave a deep greenish black color.

This was, therefore, considered to be a pigment of unknown composition, probably very closely related, chemically, to the anthocyanins, from which it differs most largely in that the color in strongly alkaline solutions is red. It is very evident that it must have been either a decomposition product from some substance present in the plant resin, or a condensation product formed by the union of two or more such decomposition products.

The fact that the color was developed in a hot solution containing considerable hydrochloric acid might suggest that it had been formed by the condensation of a furfurol derivative resulting from the action of the hydrochloric acid on a sugar with some hydroxy phenol; both the sugar and the phenol being the result of the hydrolysis of resinous material. However, the general behavior of the substance, and especially its solubility in ethyl alcohol, indicate that it is more likely to have been formed directly by the decomposition of a mother-substance, which, in the living plant, is changed into a red pigment by enzyme action.

The material extracted by sodium carbonate and sodium hydroxide solutions from the amyl alcohol extract of the acidified water solution and precipitated by hydrochloric acid, consisted of small amounts of a brown humin like substance. This contained no anthraquinones, and, because of the limited amount available, was not subjected to further investigation.

The acid aqueous liquid which had been extracted three times with amyl alcohol was just neutralized with sodium hydroxide, a dark reddish purple precipitate separating. This was resinous in character and very hard to filter. Washed with alcohol and dried in air, the color remained red, but when dried in an atmosphere of carbon dioxide, it became grayish, due probably to the formation of inorganic carbonates. In either case, it failed to dissolve in aqueous or alcoholic hydrochloric acid, which, however, turned it a yellowish brown. Nitrogen was absent. On ignition, the ash was found to contain carbonates and chlorides of iron, aluminium, and sodium. This substance,

therefore, probably consisted of a mixture of salts of organic acids, together with occluded chlorides and a certain amount of the pigment described above. Considerable humin material was also present.

The neutral aqueous liquid _ remaining was distilled under diminished pressure to remove dissolved alcohol. A small portion was tested with ammoniacal zinc acetate and found to yield no precipitate. According to A. Carpenè (Gaz. chim. Ital. 5, 129, 1875.) this would indicate that no free tannic acid was present. Neutral lead acetate was then added until no more precipitation took place. The resulting precipitate, which, of course, contained a very large percentage of lead chloride, was filtered off and the lead removed from both filtrate and precipitate by the action of hydrogen sulfide.

The lead acetate precipitate yielded a deep red aqueous solution which was boiled to remove the hydrogen sulfide. On standing, the cooled solution yielded less than 0.1 gram of a substance which crystallized in white needles, yielded little or no residue when ignited on platinum and was almost entirely insoluble in cold water and only slightly soluble in hot water. On fusion with metallic sodium, it gave a negative test for nitrogen and also for sulfur; as well as a moderately strong positive reaction for halogen. The substance melted at 258° , without decomposition, and on further heating burned with a smoky flame. While the amount was too small for further investigation, it was evident that this was some organic acid.

The filtrate from the lead acetate precipitate contained large amounts of sodium salts. Since it gave no positive

reaction for sugar, it was evident that the prolonged action of the hydrochloric acid had resulted in the decomposition of this, as well as of many other substances present in the original plant extract.

Hence, this method of investigation was abandoned, and a fresh portion of the water soluble part of the 95% alcoholic extract of the drug was prepared.

(c) Approximately two liters of this extract, representing four hundred grams of the alcoholic concentrate, were treated with enough sulfuric acid to bring the actual concentration of acid up to 1%, and allowed to stand overnight. The precipitate was small, so the solution was boiled for an hour. This resulted in the formation of a bulky, dark red precipitate, which was in sharp contrast to the light yellowish ones obtained by acidification in the cold. A pigment, probably identical with the red substance described above from the amyl alcohol extract of the acidified aqueous solution, may have accounted for this change in color.

This <u>precipitate</u>, which proved to be small in actual amount, was filtered off, dried, and extracted with ether. The ether solution yielded, on extraction with alkalies and subsequent acidification, only a very small amount of emodin, a trace of chrysophanic acid and emodin-mono-methyl ether and a large amount of a brown humin like material. The ether insoluble portion was found to consist of entirely amorphous and inseparable resins.

The <u>filtrate</u> from this sulfuric acid precipitate was neutralized with boiling barium hydroxide solution, and the barium sulfate filtered off. The solution was then clarified with

alumina cream and subjected to the usual tests for sugars. About ten cc. of this filtrate with two grams of phenylhydrazine hydrochloride and three grams of crystallized sodium acetate gave, after about five minutes heating, large quantities of a crystalline osazone. Under the microscope, the crystalline form was seen to be that of dextrosazone. The identity of this substance was confirmed by recrystallization from hot 50% alcohol, which yielded characteristic needles melting with decomposition at 205-208° C.

A second portion of the clarified sugar solution was made up to 500 cc, and the rotation measured at 20° and at 25° C. The resulting readings, (+0.85° and +0.82°, respectively) were too nearly alike to mean anything. So an effort was made to determine the amount of sugar actually present in the solution by the copper reduction method of Defren. 5 cc. portions of the solution gave 0.2816 and 0. 2821 grams of CuO, respectively. Calculated as dextrose, this would be equal to about 25.2 mg. of sugar in one cubic centimeter, or a 2.5% solution. Substituting this value in the formula: $(\alpha)^{20°} = \frac{100\,a}{1 \times c}$; where "a" equals the observed rotation, in this case, the reading of the saccharimeter times 0.3468; "1" equals the length of the tube; and "c", the percentage of sugar in the solution; we have:

$$(\alpha)^{20°} = \frac{100 \times 2.5 \times 0.3468}{2.52 \times 20} = 17.34$$

Since fructose is equal in reducing power to only 91.5% of its weight of dextrose, any fructose present would increase the value of "c", and so decrease the value of $(\alpha)^{20°}$ below that calculated. Since the value for dextrose. i.e. $(\alpha) = +53$, is much higher than that obtained, we may conclude that this sugar

consists of a mixture of dextrose and laevulose or invert sugar, with the dextrose present in the larger amounts. An attempt was made to prepare a methyl-phenyl-laevulosazone but no crystals could be obtained.

Since very large quantities of the drug were to be worked up for the emodin present, and time was limited, it was considered advisable merely to treat the remainder of the water extract with sulfuric acid to a concentration of two per cent, and allow it to stand until no further precipitation took place. The precipitate was filtered off, washed with water until free from acid, dried at room temperature, and extracted in a Soxhlet apparatus successively with petroleum ether, ether, and chloroform. These extracts were united for the sake of ease of investigation, with the similar ones obtained from the hydrochloric acid precipitate described above. The entire amount of this material obtained from forty pounds of the drug did not exceed three grams, and a large proportion of this consisted in humin material, insoluble in organic solvents.

On distillation, the <u>petroleum ether</u> extract yielded almost no residue.

The <u>ethyl ether</u> extract was shaken successively with ammonium carbonate, sodium carbonate and sodium hydroxide solutions in the manner described in greater detail later on. On acidification with hydrochloric acid, followed by shaking out with ether, the ammonium carbonate solution yielded only a small amount of a reddish resin. The acidified sodium carbonate extract yielded about 0.5 gram of emodin; while the sodium hydroxide solution was only fairly deep red in color, and on acidification

yielded only about 0.1 gram of a substance forming, on recrystallization from hot alcohol, in the golden plates characteristic of a mixture of chrysophanic acid and emodin-mono-methyl-ether. The sodium hydroxide insoluble fraction yielded practically no residue on distillation of the ether.

The chloroform extract of the precipitate was very small in amount, and consisted entirely of resinous materials.

The filtrate from the barium sulfate was concentrated under diminished pressure, and the syrup treated with strong alcohol, in an effort to cause the precipitation of some glucosidic substance. A very large amount of inorganic salts, largely potassium chloride, separated out, together with considerable quantities of the sugar described above; but the material remaining in alcoholic solution was resinous in character, and nothing further of a crystalline nature could be isolated.

## (2) The Water Insoluble Extract.

### (a) Preliminary experiments: Steam Distillation.

I. A portion (50 grams) of the resin precipitated by pouring the concentrated alcoholic extract of the drug into water was mixed with a small amount of water and distilled with steam. This distillation was continued during the working hours of the day for a period of four weeks. The distillates were collected and shaken with four successive portions of ether. The residues from these ether solutions of the first few fractions had a very pronounced odor and an oily appearance. Altho the total amount obtained was only a few drops, it was evident that an essential oil was present. Later, increasing quantities of a yellowish waxy solid were obtained. This was completely soluble in alkali, and seems to have consisted of fatty acids volatile in steam, mixed with small amounts of hydroxy-methyl-anthraquinones.

Attempts were made to separate these substances by shaking out the ether solutions with alkalies of different strengths, i.e. ammonium carbonate, sodium carbonate and sodium hydroxide. But the amounts obtained were so very small that nothing more definite could be ascertained than that there was some sodium carbonate soluble and some sodium carbonate insoluble material present.

So a second distillation was carried out with another portion of the resin. This was followed by similar attempts at purification, which yielded, in the sodium carbonate soluble fraction, only small amounts of fatty acids; while the sodium carbonate insoluble, sodium hydroxide soluble fraction yielded an hydroxy-methyl-anthraquinone melting at 196-197° .

The total solid material amounted to about 0.1 gram, and was too small for further investigation.

The aqueous layer remaining in the flask after the steam distillation of the resin was filtered from the insoluble residue. This filtrate was treated with lead acetate, the precipitate filtered off and suspended in water, and the lead removed from both filtrate and precipitate by the action of hydrogen sulfide.

The lead acetate precipitate when treated in this manner and the aqueous solution so obtained concentrated under diminished pressure yielded only resinous material.

The filtrate from the lead acetate precipitate, after the removal of the lead, was concentrated to a small volume. A portion of the concentrate, when treated with Molisch's reagent, gave a positive test for sugar. Fehling's solution was reduced very readily, and a positive reaction was obtained with the orcein-phosphoric acid reagent for laevulose. An osazone was prepared from this solution in the usual manner. It was found to have the crystalline form of dextrosazone, and to melt, after recrystallization from 50% alcohol, at 204°. An attempt to prepare methyl-phenyl-laevulosazone failed, probably because of the impurity of the sample. It seems certain, however, that this sugar was either invert sugar or a mixture of dextrose and laevulose. This must have resulted from the hydrolysis of a glucoside, because any sugar originally present in the alcoholic extract would have been in the water soluble portion of that extract. What the substance in combination with the sugar was, is doubtful, but it is at least possible that this may have been an hydroxy-methyl-anthraquinone.

(b) Analysis proper: Water Insoluble Resin.

The bulk of the water insoluble resin was dried at a temperature not exceeding 50-55° C. It was then placed in a continuous extraction apparatus and exhausted successively with petroleum ether, ethyl ether, and chloroform. Since further extraction with ethyl acetate and alcohol yielded only resinous substances from which nothing definite could be separated, the extraction of the larger part of the resin was carried no further than the chloroform.

(I) The first fractions of the <u>petroleum ether extracts of</u> this resin were deep brown in color, and yielded a resin on standing which appeared to be mostly fat. The material extracted later was deep yellow in color, and, on standing, these solutions precipitated a yellowish solid.

A. Precipitated Matter.

This solid was filtered off and recrystallized many times from alcohol. The crystals so obtained were beautiful, glistening, golden yellow plates, microscopic in size. It was soon observed that their melting point was not constant, but that repeated recrystallization did not raise it above 170°. The substance was insoluble in sodium carbonate, and soluble in sodium hydroxide with a deep red color. This fact, coupled with its behavior on recrystallization and its general appearance led to the belief that it was a mixture of chrysophanic acid and emodin-mono-methyl-ether.

So a separation was attempted--first by the method used by Tutin (Jour. Chem. Soc. 99, 955-56.) A portion (about 0.3gram) of the substance was dissolved in cold concentrated sulfuric

acid and slowly heated to 160°C, in order to demethylate it. The deep red solution was then allowed to cool to room temperature and poured into a large volume of water. This resulted in the formation of a milky deep yellow emulsion or colloidal solution. After cooling it was shaken with chloroform which extracted only about half of the coloring matter. The remainder seemed much more soluble in the aqueous sulfuric acid solution than in the chloroform, indicating that some sulfonic acid had been formed.

The part extracted with chloroform was shaken with 5% sodium carbonate, which removed very small amounts of emodin, identical with the product described later. The chloroformic extract was then shaken with 2% sodium hydroxide until no more color was removed. The sodium hydroxide solution was acidified in the usual way, and again shaken out with chloroform, the chloroform distilled off, and the residue recrystallized from alcohol. This gave deep golden yellow plates, melting at 185-186 C--not quite pure chrysophanic acid.

Since this method of separation of the emodin-mono-methyl-ether from the chrysophanic acid was not considered entirely satisfactory, an attempt was made to use the method of Oesterle. (Arch.d. Pharm. 243, 434.) About 0.5 gram of the material was dissolved in dry benzene, one gram of anhydrous aluminium chloride was added, and the mixture refluxed on the steam bath for four hours. The solution formed was reddish purple, rather than the deep blue described by Oesterle. On standing for several hours after cooling, however, a deep blue precipitate settled out, and the liquid above it was red. The benzene was distilled

off, and the residue treated with very dilute hydrochloric acid. This formed an emulsion or a sort of colloidal solution from which oily drops were seen to separate and float on the surface. The whole was about the color of powdered alizarin.

After heating on the water bath for about fifteen minutes, this color, which was probably due to the formation of some intermediate product, possibly an aluminium salt, disappeared, and a flocculent yellow precipitate formed. This precipitate was filtered off, taken up with dilute sodium hydroxide, and again precipitated with hydrochloric acid, and the dyestuff shaken out with chloroform. The chloroform solution was washed with 3 to 5% sodium carbonate solutions until the washings were colorless. The amount of carbonate soluble material so obtained was small in comparison to the same fraction obtained from the sulfuric acid purification. On treating the washed chloroform solution with two to five per cent sodium hydroxide some of the yellow coloring matter failed to be extracted, even after washing from twenty-five to thirty times and allowing it to stand in contact with the alkali overnight. Evidently, some neutral substances were formed during the demethylation process.

The sodium hydroxide solution so obtained was treated with hydrochloric acid, and again taken up by shaking with chloroform; the chloroform was distilled off, the residue taken up with dry chloroform to remove the precipitated sodium chloride, and recrystallized many times from a mixture of ethyl acetate and alcohol. Beautiful golden brown plates were obtained, melting, after drying over calcium chloride and sodium hydroxide in a vacuum dessicator, at 175° C.

Sublimation of this product over a sand bath yielded orange plates melting sharply at 189° C-- approximately pure methoxyl free chrysophanic acid. The yield was very poor and the method long, tedious, and very unsatisfactory.

B. Study of Direct Extraction.

The part of the petroleum ether extract remaining in solution after the removal of the precipitate described above was evaporated to dryness and taken up with ethyl ether. An attempt was made with a portion of this solution to effect a separation of its constituents by shaking successively with ammonium carbonate solution, then with sodium carbonate, and finally with sodium hydroxide. But the emulsions which resulted from this method of treatment refused to separate, even on long standing. A small amount of emodin was, however, obtained from the latter portions of the sodium carbonate extract. This type of separation was, on the whole, considered inapplicable to this particular solution.

C. Saponification.

The remaining residues from the petroleum ether extract of the drug (80 grams from 25 pounds of the drug ) were saponified by refluxing in the usual way with alcoholic potash. The alcohol was distilled off, and the residue mixed with sand, dried, and extracted in a Soxhlet with absolute ether. For some unknown reason, however, the potassium soaps seemed to dissolve, or rather to form a colloidal solution in the ether. At first it was supposed that this was due to the presence of moisture or of unsaponified fat, so the ether was removed and the material refluxed with a fresh portion of alcoholic potassium hydroxide.

But, altho the carefully dried residues obtained after removing the alcohol from this solution contained comparatively large amounts of free alkali, the ether extracts continued to be very dark red in color. The red material extracted was not precipitated by the addition of other nonmiscible solvents. It was found, however, that if the residues from the saponification were treated directly with the dry cold ether, the emulsification was in a large measure prevented, and a certain amount of phytosterol, mixed with a much smaller amount of hydrocarbon, was extracted. So the entire mass was treated in the cold with two or three times its bulk of ether, and the ether extracts so obtained filtered thru dry filters. These extractions were repeated sixteen times.

The ether solutions so obtained were shaken with water to remove traces of alkali; the ether was distilled off, and the residues taken up with 95% alcohol. They yielded, on recrystallization from boiling alcohol, a fairly high percentage of phytosterol, mixed with small amounts of a hydrocarbon. This latter was practically insoluble in alcohol. hot or cold, but seemed to be capable of dissolving a certain amount of the phytosterol.

After twenty recrystallizations, the phytosterol was obtained in water white solution, most of the color going into the hydrocarbon fraction. The yield of the snow white plates amounted to 1.15 grams. Their melting point, which was 132°-133° C, remained unchanged when they were mixed with the crystals of a phytosterol isolated during a similar investigation of cascara. On treatment with acetic anhydride and a few drops of sulfuric acid a red color developed. This changed thru purple to

blue, and finally, after standing for some time, to green and brown. (Liebermann-Burchard Reaction, Ber. 18, 1804, 1885.) On combustion, the results obtained were at first inconsistent with the formula $C_{20}H_{34}O$, as is shown in the table below. But since a compound was found to have been described in the literature corresponding more nearly to the formula: $C_{20}H_{34}O.H_2O$; (Tutin, J. Ch. Soc. 97, 1, 1910) we heated portions of this phytosterol to constant weight at 97°C with the results shown in the first table. It was evident that, while the moisture content of the samples was not uniform, the range was comparatively small. So the results of the combustion made at first were recalculated on the basis of the weight of the dry material, and on combustion of another sample results corresponding to the theoretical formula were obtained.

Table I.

| Wt. Sample | Dried at 97' | Loss in wt. | % water. |
|---|---|---|---|
| 0.1350 | 0.1319 | 0.0031 | 2.35 |
| 0.1486 | 0.1453 | 0.0035 | 2.33 |
| | | | Average: 2.34. |
| Theory, $C_{20}H_{34}O.H_2O$ | | | 5.8 |

Table II.

| Wt sample. | Carbon dioxid | Water | %C | %H. |
|---|---|---|---|---|
| Theory | -----= | - ---- | 82.8 | 11.7 |
| 0.1500 | 0.4481 | 0.1632 | 81.5 | 12.1 |
| 0.14655 (recalc.) | 0.4481 | 0.15985 | 82.4 | 12.2 |
| 0.1319 | 0.4021 | 0.14185 | 83,1 | 12.0 |

The phytosterol acetate was prepared by refluxing with acetic anhydride and fused sodium acetate. It melted at 122°C. and the melting point was unchanged when it was mixed with the acetate of a similar phytosterol prepared from cascara.

This substance was, therefore, evidently the phytosterol rhamnol, identical with that isolated by the present authors from cascara and previously described by Jowett. (Proc. Am. Pharm. Assoc. 52, 288, 1904)

The hydrocarbon found associated with the phytosterol was present in extremely small amounts, and was so thoroly mixed with small portions of the phytosterol, and with soaps carried over mechanically in the ether solution, that separation in an entirely pure state was impossible. It was evident, tho, that it liquefied below the boiling point of alcohol and was solid at room temperature. It was insoluble in concentrated sulfuric acid, sulfonated with fuming sulfuric acid, and took up a comparatively small amount of bromine. The amount obtained did not permit of further investigation.

The alkaline residues remaining after the extraction of the saponified material with ether were taken up with water, acidified, extracted with ether, and the ether solution shaken twenty times with 5% sodium carbonate and finally twice with 2% sodium hydroxide.

The sodium carbonate solutions were deep reddish brown, at first, later becoming pink. They tended to emulsify very badly while in contact with the ether. So, after acidification with hydrochloric acid, they were again shaken out with ether, the ether distilled off and the residues taken up with dry ether and dried further by standing over anhydrous sodium sulfate for twenty four hours. The fatty acids present (approximately 60 grams in 170 grams of ether) were then esterified by refluxing with 100 grams of a 2.5% solution of dry hydrochloric

acid in absolute ethyl alcohol. After twelve hours on the steam bath, the ether solution of the esters so obtained was shaken twice with water to remove the alcohol. It was then shaken with ammonium carbonate solution, which removed practically nothing. Next, it was washed with 5% sodium carbonate until the washings ceased to be colored. This latter treatment removed some emodin, together with fairly large amounts of brown resinous material.

The ether solution of the fatty acid esters was then washed with 0.5% sodium hydroxide. This removed only traces of an unidentified anthraquinone dyestuff.

The ether was distilled from the solutions of the fatty acid esters remaining after these washings; and the esters so obtained were fractionated, first under atmospheric pressure, and later at 20 mm.

After traces of ether had been removed, the mixture boiled for a few minutes at 77°C, indicating the presence of a very small amount of ethyl acetate. The next fairly constant boiling point was between 90° and 95° C, approximately the boiling point of ethyl iso-butyrate. Then the temperature rose suddenly to 140°-145°, where a few drops distilled over. This would indicate the presence of small amounts of ethyl propionate or ethyl valerianate.

After this the temperature rose suddenly to 200° C. At this point the distillation apparatus was connected with a vacuum pump, and the pressure reduced to 20 mm. The boiling point then rose suddenly to 208°-210°, and a fraction of 5.28 grams was collected. At 220°-222° another fraction of 8.47 grams distilled over, and from 226 to 235°, a third fraction of 4.03 grams. Then there was a

sudden rise in temperature, and at 255-265° C another fraction of 5.35 grams was collected. At 265° a crystalline solid began to collect in the condenser tube. While the boiling point remained constant at this point, 2.52 grams of this substance were collected.

This solid ester was insoluble in cold alcohol, and crystallized in irregularly formed plates from hot alcohol. Repeating the fractionation resulted in very little change in the boiling points of the various fractions and in the proportionate yields obtained.

Weighed samples of the various esters were saponified by boiling with measured amounts of half normal alcoholic potassium hydroxide, according to the method of Koettstorfer as given in Sherman's "Organic Analysis" 2nd Ed. pp 144-148. The saponification equivalents were then determined by titration with standard hydrochloric acid. The results are tabulated below. It is evident that the samples of the solid esters taken were not uniform.

| B.P. Ester. | Wt. Ester taken | No. cc. HCl, N.F.O. 4690 | Sap. Equiv. | Sap. No. |
|---|---|---|---|---|
| 208-210° C | 1.93475 | 13.95 | 297.7 | 188.5 |
| | 1.55960 | 10.6 | 316 | 181.6 |
| 220-222° C | 3.0848 | 22.2 | 296 | 189.5 |
| | 2.2176 | 15.6 | 295.9 | 189.7 |
| | 3.15610 | 23 | 293.2 | 191.8 |
| 235° C | 1.15205 | 8 | 305.2 | 183.8 |
| 255-265° C (liquid) | 2.46430 | 15.1 | 346 | 162.1 |
| | 2.5842 | 16 | 346.6 | 161.8 |
| 265° C (solid) | 1.5691 | 7.5 | 438.8 | 127.7 |

An excess of hydrochloric acid was added, after the titration of the unsaponified alkali, and the fatty acid emulsions were

heated on the water bath to remove the alcohol present. After cooling, the cakes of fatty acid formed were filtered off, washed with water, taken up with small amounts of ether, and the ether evaporated off. The acids were then dried to constant weight at 97° C, in order to remove occluded hydrochloric acid. Weighed samples were then dissolved in hot neutral alcohol, and the neutral equivalents determined. The results are given in terms of twentieth normal alkali, together with the melting points of the acids.

| B.P. Ester. | Wt. Acid taken. | No. cc. NaOH 0.05264 N. | Neut. Equiv. | Acid No. mgs. KOH | M.P. Acid. |
|---|---|---|---|---|---|
| 208-310° C | 0.2599 g | 15.5 | 318.5 | 176.6 | 45° C |
| | 0.3531 | 21.2 | 316.4 | 177.3 | |
| 220-222° C | 0.3002 | 19 | 298 | 188.3 | 42° C |
| | 0.4187 | 26.7 | 300.2 | 186.9 | |
| 235° C | 0.2814 | 17.1 | 312.5 | 179.5 | 43° C |
| 255-265° C | 0.3687 | 19.85 | 352.9 | 159 | 28° C |
| (liquid) | 0.3998 | 21.6 | 351.8 | 159.6 | |
| 265° C | 0.2023 | 8.15 | 471.5 | 119 | 32° C |
| (solid) | 0.15205 | 6.15 | 469.6 | 119.7 | |

The iodine numbers of the various fatty acid fractions were then determined according to the method of Hanus, as adopted by the Association of Official Agricultural Chemists. (Sherman, Org. Anal. Ed. 2, 153-157) The results are listed below:

| B.P.Ester. | Wt. Acid taken. | No. cc. $Na_2S_2O_3$ 0.09724 N. | % $I_2$ Absorbed. |
|---|---|---|---|
| 208-210°C | 0.3083 g | 11.85 | 47.21 |
| | 0.25345 | 9.7 | 47.24 |
| 222°C | 0.4256 | 20.85 | 59.2 |
| | 0.4367 | 21.21 | 60.2 |
| 235°C | 0.2332 | 7.15 | 30.1 |
| | 0.3390 | 8.85 | 32.1 |
| 255°C | 0.4020 | 15.3 | 46.9 |
| (liquid) | 0.3589 | 13.5 | 46.4 |
| 265°C | 0.29125 | 18.2 | 76.8 |
| (solid) | 0.3000 | 18.75 | 77.0 |

The part of the 220° fraction remaining after these investigations was recrystallized several times from 90% alcohol, washed with 90% and with 70% alcohol, and finally recrystallized from boiling absolute alcohol. This yielded a product melting at 62°C, and having a neutral equivalent of 269.5---corresponding to a mixture of palmitic and stearic acids. Arachidic acid was, therefore, absent. No material was available for further investigation of the other fractions.

The saturated acids present are, therefore, palmitic and stearic, the stearic acid in the larger amount. The unsaturated erucic acid, $C_{22}H_{42}O_2$ (mol. wt. 388, m.p., 33°-34°, iodine number 75.15, B.P. ethyl ester above 360° at 760 mm, without decomposition) probably forms the chief constituent of the 255-265° fractions. Small amounts of fatty acids of still higher molecular weights and unsaturated fatty acids of lower molecular weights, possibly of the linolic series, are undoubtedly present.

The information gained with regard to the properties of these fatty acids is summed up in the following table:

| B.P. Ester. | M.P. Acid. | Sap. No. (Av.) | Sap. Equiv. (Av.) | Neut. Equiv. | Acid No. (Av.) | I, No. (Av) |
|---|---|---|---|---|---|---|
| 208-210° | 45° C | 185 | 300-307 | 317 | 177 | 47.2 |
| 220° C | 42° C | 190 | 295 | 299 | 187 | 59.7 |
| 235° C | 43° C | 183.8 | 305 | 312 | 179.5 | 31. |
| 255° C (liquid) | 28° C | 162. | 346.3 | 352.5 | 159.3 | 46.7 |
| 265° C (solid) | 32° C | 127.7 | 439. | 470. | 119. | 76.9 |
| 220° C (recryst) | 62° C | -- | -- | 269.5 | -- | --- |

The sodium hydroxide soluble, sodium carbonate insoluble fraction extracted by ether after decomposing the saponified petroleum ether extract with acid was acidified with hydrochloric acid in the usual manner. The material precipitated by the acid was extracted with ether, the ether distilled off, and the residue recrystallized many times from boiling alcohol, in which it was only slightly soluble. The alcoholic solutions were golden yellow and yielded about two grams of a substance which crystallized in beautiful golden spangles, aggregating in tree like forms. They melted sharply at 196° C.

An acetyl derivative was prepared by boiling this substance for six hours with acetic anhydride and fused sodium acetate. The mixture was poured into boiling water to decompose the excess of anhydride, and the precipitated material recrystallized from boiling 95% alcohol. This gave beautiful, glistening, greenish yellow hexagonal plates, melting sharply at 204° C. This corresponds to the melting point reported in the literature for acetyl chrysophanic acid.

Combustion of this substance by the ordinary method of Liebig gave results which were too low, due to the fact that some of the substance sublimed unchanged thru the red hot cupric oxide and condensed in the forward cooler end of the tube. Using the method described by Levene and Bieber, (J. Am. Ch. Soc. 40, 460-463, 1918.) with a catalyst of cerium oxide asbestos, made by igniting purified asbestos which had been previously suspended in a concentrated solution of cerium nitrate and partially dried on the steam bath, in a current of oxygen; the following results were obtained:

| Wt. Substance. | Wt. Carbon Dioxide | Wt Water | %C | %H |
|---|---|---|---|---|
| 0.1011 g | 0.2616 | 0.0352 | 70.6 | 3.95 |
| 0.1207 | 0.3166 | 0.0400 | 71.4 | 3.7 |
| Theory, $C_{15}H_{10}O_4$ | | | 70.9 | 3.9 |

This was considered to complete the proof that this substance was methoxyl free chrysophanic acid.

The sodium hydroxide insoluble fraction of the petroleum ether extract which had been held in the aqueous layer after the saponification yielded only small amounts of the phytosterol described above.

The ethyl ether extract of the original water insoluble resin amounted to eighteen liters. This was shaken out seven times with three times its bulk of approximately 8% ammonium carbonate, 378 liters in all.

A. The ammonium carbonate solutions so obtained were neutralized by siphoning into a slight excess of concentrated

hydrochloric acid, and the material precipitated by the acid again brot into solution by shaking out with ether. The ether extracts were separated, the ether distilled off, and the residues treated first with warm glacial acetic acid. The resinous matter brot into solution was separated by filtration, and the residue dissolved in boiling alcohol. This solution was filtered, allowed to cool, and again filtered; the precipitated matter being treated once more with warm glacial acetic acid. The insoluble fraction was again brot into solution in hot alcohol, filtered, allowed to cool, and the precipitated material separated. This was recrystallized several times from boiling glacial acetic acid. On final recrystallization from boiling alcohol, it formed in beautiful orange needles, melting at 250° and having the characteristic form of the emodin which is described in detail later on. It was found to be in every way identical with this substance. Nothing crystalline could be isolated from the brown resinous material which composed the fraction of the ammonium carbonate extract readily soluble in glacial acetic acid and alcohol.

B. After the ammonium carbonate washings from the original ether extract of the water insoluble resin had become colorless; the treatment with this solvent was discontinued, and a 5% solution of sodium carbonate substituted. The first two washings with this solvent yielded very deep red alkaline solutions, which, on acidification with hydrochloric acid, yielded deep orange precipitates. On subsequent washings, a quantity of less soluble material showed a tendency to separate in the layer between the ether and the alkali. So, after six washings, amounting to about 216 liters, the treatment with carbonate was discontinued, and

the ether solutions separated as sharply as possible from the emulsions.

These ether solutions were then shaken out with 2% sodium hydroxide, which was deeply colored at first, but became colorless after two washings. In order to make sure that no sodium carbonate soluble material was present in this fraction, it was saturated, before neutralization, with carbon dioxide. This precipitated a quantity of crystalline material, which is described under the heading of the sodium hydroxide soluble fraction. The filtrate from these crystals was acidified, shaken out with ether, and added to the sodium carbonate soluble fraction.

This sodium carbonate soluble fraction, (about 216 liters) was acidified in the usual manner by siphoning into concentrated hydrochloric acid, and the dissolved ether removed by a current of air. The deep orange colored precipitate was then filtered off, dried on the filter papers in a hot closet, the filters placed in a Soxhlet apparatus and exhausted with absolute ether. The ether was distilled off, and the residues obtained were treated with warm glacial acetic acid. The resinous material removed by this first treatment was separated by filtration, and the less soluble fraction recrystallized from boiling glacial acetic acid. This yielded 16-18 grams of the beautiful needle shaped crystals of emodin. These, on further recrystallization from hot alcohol, melted at 253° C. On combustion, by the method used for chrysophanic acid, the following results were obtained:

---

| Wt. Substance | Wt. Carbon dioxide | Wt.water | %C | %H. |
|---|---|---|---|---|
| 0. 09 g. | 0.2204g | 0.0318 g | 66.8 | 3.9 |
| 0.1433g | 0.3564 g | 0.0496 | 66.7 | 3.88 |
| Theory $C_{15}H_{10}O_5$ | | | 66.7 | 3.7 |

An acetyl derivative was prepared by refluxing with acetic anhydride and fused sodium acetate. The excess anhydride was decomposed by pouring into boiling water, and the precipitated material recrystallized from hot 95% alcohol, and finally from ethyl acetate. The part least soluble in alcohol yielded beautiful lemon yellow needles, melting sharply at 197° C. This corresponds to the acetyl emodin described in the literature.

A benzoyl derivative was next made by the Schotten-Baumann method, sufficient benzoyl chloride being used to make the mixture distinctly acid at the end of the process. The precipitated material was first extracted with 50% alcohol to remove the benzoic acid, and then recrystallized from hot 95% alcohol. The product so obtained melted at 224° C, corresponding to the dibenzoyl derivative of emodin described in the literature.

Nothing crystalline except emodin could be isolated from the sodium carbonate soluble fraction.

The sodium hydroxide soluble fraction, precipitated by saturating this solution with carbon dioxide, was filtered off and washed with water until free from alkali. This took a great deal of time, since the precipitated substances were crystalline in small scales, which formed an almost impervious layer on the

filter paper. This precipitate was dried on the filters in the hot closet, and the whole mass extracted in a Soxhlet apparatus with chloroform. The chloroform was distilled off, and the residues recrystallized from boiling 95% alcohol. They yielded about four grams of a mixture of chrysophanic acid and emodin-mono-methyl ether, having the same properties as that previously described from the petroleum ether extract.

The ether extract of the water insoluble resin which had been washed with the various alkalies yielded, on distillation of the ether, practically no residue.

The chloroformic extract of the water insoluble resin yielded nothing on shaking with ammonium carbonate solution, while the washings with sodium carbonate were only faintly pink, as were also those with sodium hydroxide. The residues left after distillation of the chloroform were examined carefully, but the only crystalline substance which could be isolated was sodium chloride. The whole amount of this extract from about ten pounds of the drug did not exceed one gram; so it was evident that the extraction with ether had been more thoro than in the preliminary examination, the process had been so long continued as to render some previously soluble material insoluble, or that the cold ethyl alcohol used in the percolation had not extracted some of the chloroform soluble substances in the original drug.

(III) Summary of Results.

Rumex crispus contains, therefore, in the water soluble portion of the 95% alcoholic extract of the root, very small amounts of emodin, emodin-mono-methyl-ether, and chrysophanic acid. These substances are probably in some sort of glucosidic

combination at the time of the precipitation of the resin. There are also present in this fraction; several pigments, notably one which is soluble in alcohol with a red color, is red in acid solution, greenish black when the solution is neutralized, and again red when it is strongly alkaline, some organic acids, large amounts of sugars giving d-phenyl glucosazone—probably a mixture of dextrose with invert sugar— and very large amounts of resinous material.

From the water insoluble resin, emodin; chrysophanic acid; a mixture of chrysophanic acid and emodin-mono-methyl-ether; a phytosterol, identical with the rhamnol from cascara; various fatty acids, including palmitic, stearic and erucic acids; a sugar yielding d-phenyl- glucosazone; and large proportionate amounts of resins have been isolated. Besides, the presence of an essential oil, various volatile fatty acids and glucosides is clearly indicated.

## D. Study of Cascara.

The investigation of Cascara followed, even more typically than the analysis of Rumex, the general method outlined. Since H.A.D. Jowett (Proc. Am. Pharm. Assoc. 52, 288, 1904.) has published an account of a more complete analysis of this drug, and our work was designed primarily as a preparation of the anthraquinone derivatives of cascara for purposes of comparison, we will report it only in brief outline.

Five pounds of the bark in No. 40 powder were packed in a percolator, and exhausted with cold 95% alcohol. The alcohol was distilled off under diminished pressure, and the resins precipitated from the concentrated extract by pouring it into an excess of water.

### I. Water Soluble Extract.

The water soluble portion was shaken several times with each of the usual solvents, i,e., petroleum ether, ether, and amyl alcohol. Then the remaining aqueous extract was precipitated with neutral lead acetate, and finally with basic lead acetate. These precipitates were decomposed in the usual way with hydrogen sulfide, and a careful study made of the substances liberated. Only resinous matter, some of it evidently containing tannins, was isolated. The filtrate from the basic lead acetate yielded, after the removal of the lead, a sugar which gave d-phenyl glucosazone and a positive test with the orcein-phosphoric acid reagent for laevulose.

The ether and amyl alcohol extracts of this water soluble portion were shaken successively with the usual alkalies, as in the investigation of Rumex. A small amount of emodin

was isolated in a pure state from the acidified sodium carbonate extract of the ether soluble portion; but the petroleum ether extracted almost nothing, and only resins could be isolated from the amyl alcohol extract.

II. The Water Insoluble Resin.

The water insoluble resin was dried, mixed with purified sawdust, and extracted in a Soxhlet apparatus successively with petroleum ether, ethyl ether, chloroform, and alcohol.

The petroleum ether extract was freed from the solvent, taken up in ethyl ether, and this solution shaken successively with ammonium carbonate, sodium carbonate, and sodium hydroxide solutions. After considerable trouble with emulsions, these extracts were finally separated.

On acidification, the ammonium carbonate extract yielded only resinous materials.

The sodium carbonate extract contained fairly large amounts of fatty acids. An attempt was made to carry out a separation of the saturated from the unsaturated acids by extraction of the lead soaps with ether, but it was found that the process of extraction had resulted in the oxidation of some unsaturated acids to the extent that a good separation was impossible. By a process of fractional recrystallization from alcohol of different strengths, a small amount of arachidic acid was obtained in fairly pure form. There was considerable evidence, also, that the unsaturated acids belonged to the linolic series. Small amounts of emodin were also found to be present in this fraction.

The sodium hydroxide extract contained only traces of

anthraquinone containing material.

The neutral fraction remaining in the ether solution after these extractions was saponified with alcoholic potassium hydroxide solution, the alcohol removed, and the residues extracted with dry ether. This ether extract yielded a phytosterol, m.p. 132° $(\alpha)^{21^\circ}$ = 26.1 in chloroform solution; acetyl derivative prepared in the usual way, m.p. 122° C. As has been stated before, this phytosterol was proven to be identical with that isolated from the petroleum ether extract of the water insoluble resin of Rumex crispus. The fatty acids set free on acidifying the aqueous solution of the potassium soaps were combined with those from the sodium carbonate extract for purposes of examination.

The ethyl ether extract of the water insoluble resin was shaken successively with 8% ammonium carbonate, 5% sodium carbonate and 2% sodium hydroxide solutions.

The ammonium carbonate extracted only resinous material, from which no definite compounds could be isolated.

After acidification, the sodium carbonate extract yielded emodin, which, on recrystallization from glacial acetic acid, was obtained in beautiful orange needles, melting, after further recrystallization from alcohol and from pyridine, at 252° C. An acetyl derivative was prepared in the usual way and found to melt at 197° C. This was, at first, obtained in what appeared to be green plates. On close examination under the microscope, these proved to be prisms, and on recrystallization from ethyl alcohol, the substance formed in yellow prisms, appearing macroscopically, to be needles. These were identical with those formed from the acetyl derivative of the emodin isolated from

Rumex crispus. Since there was no mention in the literature of a greenish modification of the acetyl derivative of emodin, a study was made of the different possible types of crystals of this compound; and it was found that the solvent, and the temperature at which the substance was dissolved, seemed to alter the shape of the crystals considerably. Quick recrystallization from a solvent in which the substance was very slightly soluble produced the microscopic, comparatively broad crystals at first observed, while slow crystallization from a solvent like ethyl acetate gave the clusters of needle like prisms ordinarily considered characteristic of this compound.

The chloroform and alcohol extracts of the resin yielded no definite compounds.

E. Study of Aloes.

This was undertaken in order to obtain a sample of aloe-emodin for purposes of comparison. About 400 grams of Barbadoes aloes obtained from Meyer Bros., St. Louis were ground up, mixed with purified sawdust, and exhausted by percolation with cold 95% alcohol. The alcoholic extract was concentrated under diminished pressure, and finally mixed with purified sawdust and evaporated to dryness on a steam bath. After breaking up into small pieces, this residue was packed in a continuous extraction apparatus and exhausted, first with petroleum ether (B.P. 40°-60°) and then with ethyl ether.

The petroleum ether extract so obtained yielded, on distillation of the solvent, only small quantities of fats and fatty acids. Since no anthraquinone containing material was present, this fraction was not further investigated.

The ethyl ether extract was shaken successively with ammonium carbonate, sodium carbonate and sodium hydroxide solutions.

After acidification, followed by shaking out with ether, and distillation of this solvent, the ammonium carbonate solution yielded only a very small amount of a dark reddish brown resin with an odor strongly characteristic of the drug.

The sodium carbonate solutions were only slightly pink, and since it was evident that the anthraquinone derivative causing this coloration was only very slightly soluble in the carbonate, the extraction with this solvent was repeated only twice. The first fraction yielded, on acidification, a very small amount of resinous material; while the second fraction evidently contained minute quantities of aloe-emodin. This was, therefore, examined

in connection with the sodium hydroxide soluble fraction.

The first extraction with sodium hydroxide solution removed most of the coloring matter from the ether, and a second shaking out completed the process. On acidification, this extract yielded a small amount of aloe-emodin, identical in every respect with the product described below.

Since the total amount of material removed from the original resin by the ether and petroleum had been extremely small, and the best evidence in the literature pointed to the fact that most of the aloe-emodin was present in the drug in the form of a glucoside, there seemed to be good reason to think that much larger quantities of aloe-emodin might be obtained by hydrolysis of the resin.

The residue which had been exhausted with petroleum ether and ethyl ether was taken from the extraction apparatus, treated with alcohol to remove the resinous material from the sawdust, the alcoholic extract concentrated, and the concentrate suspended in a 2% aqueous solution of sulfuric acid.

This acidified suspension was placed in two four-liter flasks and covered with a layer of benzene about two inches thick. The flasks were connected with reflux condensers and placed on the steam bath. As the benzene layers became saturated with substances set free by hydrolysis, they were drawn off and replaced by fresh benzene. In this way, the products of the reaction were removed as the hydrolysis proceeded. It was found, however, that this type of hydrolysis was too slow to be very practicable for a large scale analysis.

The benzene extracts so obtained were deprived of the solvent

and the residues treated with ethyl ether. These ether solutions were shaken successively with 8% ammonium carbonate, 5% sodium carbonate, and 2% sodium hydroxide solutions.

The ammonium carbonate extracts, on acidification, yielded a yellowish precipitate which proved to be crystalline. This was taken into ether solution, and the solvent distilled off. On recrystallization from hot water, yellow scales were formed, which proved, under the microscope, to consist of aggregations of small crystals of the type characteristic of cinnamic acid. The yellowish tint was hard to remove; but, on repeated recrystallization from petroleum ether, the substance was obtained in almost colorless form. Its melting point was 132°C. On oxidation with acid permanganate, it yielded benzaldehyde. The p-nitro derivative was prepared according to the directions given by Mulliken (Identification of Pure Organic Compounds, Vol. I, p 83, John Wiley and Sons, N. Y., 1908). This softened at about 265°C and melted at about 285°. The presence of cinnamic acid was therefore considered to have been demonstrated.

The sodium carbonate extraction was repeated only four times since this alkali seemed to be causing the precipitation of some slightly soluble substance. After acidification and extraction with ether in the usual way, the residues from the ether solution yielded small amounts of aloe-emodin identical with the product described below.

The sodium hydroxide extracts were deep red. On acidification, a dark orange yellow precipitate was formed. After repeated alternate recrystallization from ethyl alcohol and glacial acetic

acid, this was finally obtained in dark orange colored needles, melting at 224° C. On acetylation with acetic anhydride and fused sodium acetate, followed by decomposition of the excess anhydride with boiling water, and recrystallization of the precipitated acetyl derivative from 95% alcohol, this was obtained in lemon yellow needles, melting at 175°-176° C; corresponding to the diacetyl aloe-emodin described in the literature.

A benzoyl derivative was made by the Schotten Baumann reaction This, after purification by solution in chloroform, followed by precipitation with alcohol, formed in microscopic lemon yellow needles, melting at 237-238° C. This was the tri-benzoyl aloe-emodin described by Tutin and Naunton. (Pharm. Jour. 91, 836 1913.) The identity of the aloe emodin was therefore considered to have been established, and the fact that it was not identical with the emodin from Rumex to have been proven.

## IV. Recapitulation of Results.

A. Comparison of the Properties of the Emodins of Rumex, Cascara and Aloes.

The emodin of Rumex crispus forms in orange red needles, melting at 250-255° C when recrystallized from alcohol or pyridine gives an acetyl derivative forming lemon yellow needles which melt at 197° C, and a dibenzoyl derivative which melts at 224° C. This emodin is soluble in alcohol, glacial acetic acid, ether, benzene and chloroform, and easily soluble in the alkali carbonates and hydroxides; properties which check, in every way, those of the emodin isolated from cascara. The identity of these emodins has further been proven by the fact that the melting point remains the same when the two compounds are mixed. This would indicate, moreover, that the emodin of R. crispus is also identical with that from R. ecklonianus (Tutin and Clewer, J. Ch. Soc. 97, 1, 1910.), with that from rhubarb (Tutin and Clewer, J. Ch. Soc. 99, 946, 1911.), and probably with that described by various authors from frangula.

It differs from the emodin of aloes, and consequently from that of senna; (Tutin, J. Ch. Soc., 103, 2006, 1913.) in that this latter compound melts at 224° C, forms an acetyl derivative melting at 177° C, and when benzoylated under the same conditions with which the emodin of Rumex forms a dibenzoyl derivative melting at 224° C, forms a tribenzoyl aloe-emodin melting at 235° C Aloe-emodin is also much less soluble in cold solutions of the alkali carbonates, and in cold alcohol. The chief difference in structure between the two isomers lies in the fact that aloe-emodin has two hydroxyl groups in the ring and one on the side

chain, while emodin has three hydroxyls in the ring and none on the side chain.

While no chrysophanic acid has been prepared from a drug other than Rumex during this investigation; since the properties of the compound from Rumex crispus check in every way those of the compound from rhubarb (loc. cit.) and from Rumex ecklonianus, (loc. cit.), which was described as forming in golden yellow spangles melting at 197°C and yielding an acetyl derivative forming greenish yellow plates which melt at 204°C, are very slightly soluble in ether and cold alcohol and more soluble in ethyl acetate and chloroform; there is very conclusive evidence that the compound from Rumex crispus is identical with those from the other drugs.

The mixture of chrysophanic acid and emodin-mono-methyl-ether isolated from R. crispus has properties identical with those described for a similar mixture from a different source by Oesterle and his coworkers. (Arch. d. Pharm. 248, 476, 1910, and 249, 445, 1911.)

The combination of hydroxy methyl anthraquinones present in Rumex crispus is not the same as that described for any other family of plants, altho it agrees exactly with that of Rumex ecklonianus. Cascara contains only emodin and an iso-emodin; senna, aloe-emodin and rhein; aloes, aloe-emodin; and rhubarb, not only rhein, emodin, and emodin-mono-methyl ether, but also chrysophanic acid, aloe-emodin, and rheinolic acid. There seems, however, to be some possibility that Rhamnus frangula may contain the combination of emodin, emodin mono-methyl-ether and chrysophanic acid which is present in the Rumex.

## Summary and Conclusions.

(1) The following substances are present in the material extracted from the root of Rumex crispus by cold 95% alcohol:

(a) Soluble in water: Small amounts of emodin and a mixture of emodin-mono-methyl-ether and chrysophanic acid; a pigment which is probably related to the anthocyanins; sugars yielding d-phenyl-glucosazone and having properties indicating the presence of laevulose and invert sugar as well as dextrose; besides organic acids and much resinous material. It is very probable that some of these substances are present in the plant in the form of glucosides.

(b) Insoluble in water: Emodin, chrysophanic acid, a mixture of chrysophanic acid and emodin-mono-methyl-ether, a phytosterol, palmitic, stearic and erucic acids, together with unsaturated fatty acids of lower and saturated fatty acids of higher molecular weights, a small amount of an unidentified hydrocarbon, probably a terpene, an essential oil, and a large percentage of resinous material. The presence of glucosides is clearly indicated.

(2) The emodin isolated from Rumex crispus is identical with that from cascara (Rhamnus purshiana) and the phytosterol of Rumex crispus is identical with the rhamnol isolated from cascara.

(3) The yield of emodin from the dry root of Rumex crispus amounts to about 0.1% of its weight, and that of chrysophanic acid is somewhat less. This compares favorably with the yields from more expensive drugs, and it is probable that, if resinification could be prevented during the various extractions,

much higher yields could be obtained.

# VITA.

The writer was born near Woodsfield , Ohio, September 13, 1893. She attended the public schools in that town, graduating from the Woodsfield High School in 1911. In the fall of 1911, she entered the College of Liberal Arts at Ohio University, Athens, Ohio; where she remained one year. In 1912, she matriculated at Monmouth College, Monmouth, Illinois, from which institution she was graduated with the degree of Bachelor of Science in Chemistry in 1914, receiving the nomination of the college faculty for a scholarship in the Graduate School of the University of Illinois for the year 1914-1915. She completed the work for the degree of Master of Science in Chemistry at the University of Illinois in 1915, and taught there as a Graduate Assistant in Chemistry during the years 1915-1917. In 1917 she was granted a fellowship in Chemistry at the University of Illinois. With Dr. George D. Beal, she has published a paper on "The Qualitative Identification of Drugs Containing Emodin" (J. Am. Ch. Soc. 39, 716-725., 1917.)

I wish to take this opportunity to express my appreciation of the very valuable assistance which I have received during the entire course of this investigation from Dr. George D. Beal, under whose direction it has been carried out.